

Studies on Virus Diseases of Fishes. Epizootiology of Epithelial Tumors in the Skin of Flatfishes of the Pacific Coast, with Special Reference to the Sand Sole (*Psettichthys melanosticus*) from Northern Hecate Strait, British Columbia, Canada

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(Plates I-XI; Text-figures 1 & 2)

INTRODUCTION

SOLITARY or multiple epithelial tumors described as cutaneous warts, papillomas and hyperplastic epidermal diseases, and for which a viral etiology has been suggested or implied, have been reported in several species of European and North American flatfishes (Order: Pleuronectiformes, or Heterosomata) (Table 1). The early reports deal with isolated incidences while the more recent publications are concerned with epizootics involving hundreds of individuals in flatfish populations in the following three general areas of the Pacific coast of North America: (1) sand soles from British Columbia (Ketchen, 1953); (2) lemon and flathead soles from Washington (Pacis, 1932; Good, 1940; Chuinard, *et al.*, 1964); and (3) lemon and Dover soles from southern California (Herald & Innes, *pers. comm.*; Young, 1964).

The present paper deals with the epizootiology of the disease causing epithelial tumors in sand soles from British Columbia, and includes the histopathology of the tumors in this species and in rock soles from the same area.

DESCRIPTION OF THE TUMORS

Tumors from formalin-fixed specimens of sand and rock soles were sectioned at 4 microns and stained with the following: Harris's hematoxylin-eosin; Heidenhain's iron-hematoxylin with and without eosin; Masson's triple stain; Giemsa's stain; Heidenhain's "Azan" variant; Mayer's mucicarmine; and PAS. Formalin-fixed

tissue was also treated with osmium tetroxide (2% sol.; vapor method) and in some instances followed with Heidenhain's iron-hematoxylin.

Macroscopically, the epithelial growths in the sand sole are found in the skin of relatively young fish (1-3 years old), usually less than 20 cm. in length (Table 2, Text-fig. 2). The lesions appear as solitary or multiple, flat or raised, grayish or brownish growths of various dimensions. They occur on both sides of the fish but more frequently on the pigmented or eyed side of the body, usually adjacent to and often associated with the rays of the fins (Pl. figs. 1 & 2). In some cases, the tumors are also found in the head region, occasionally involving the *limbus corneae* and the operculum. Epithelial tumors in the rock and lemon sole are shown for comparison with the sand sole in Pl. figs. 3 & 4. The tumor in the lemon sole (Pl. fig. 4) appears to be a papilloma, the pathology of which will be described at a later date.

Microscopically, the lesions vary from a simple to an extensive hyperplasia of the epithelium, with a papillary-like structure present in some areas (Pl. figs. 7-11, 15, 19, 22). However, the over-all appearance of the growths is suggestive of a regressive process. No mitotic figures were seen and the staining reaction, in general, was weak, with some evidence of inflammatory responses and necrotic changes.

The epithelium in "normal" areas of the skin consists of stratified cells, interspersed with mucous cells (Pl. fig. 5). In an area that represents a transitional region between "normal" and hyperplastic epithelium (Pl. fig. 6), enlarged epithelial cells characteristic of the main

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TABLE 1. EPITHELIAL TUMORS IN THE SKIN OF FLATFISHES (PLEURONECTIFORMES, OR HETEROSOMATA)

| Species | No. Fish Reported | Common Name | Locality | Author |
|---------------------------------------|-------------------|-----------------------|--------------------------|---|
| <i>Hippoglossus hippoglossus</i> | 1 | Halibut | North Sea | Johnstone, 1912 |
| <i>Pleuronectes platessa</i> | 2 | Plaice | North Sea | Johnstone, 1925 |
| <i>Pleuronectes (Limanda) limanda</i> | 3 | Dab | North Sea | Johnstone, 1925 |
| <i>Solea solea</i> | 2 | Sole | North Sea | Thomas, 1930 |
| <i>Pseudopleuronectes americanus</i> | 2 | Winter Flounder | L. I. Sound | Smith, 1935 |
| <i>Parophrys vetulus</i> | Epizootic | Lemon or English Sole | Washington, California | Pacis, 1932; Good, 1940; Wellings, <i>et al.</i> , 1965; Herald & Innes, MS |
| <i>Psettichthys melanosticus</i> | Epizootic | Sand Sole | B.C., Canada, Washington | Ketchen, 1953; present paper; Wellings, <i>et al.</i> , 1965 |
| <i>Lepidopsetta bilineata</i> | 5 | Rock Sole | B. C., Canada | Ketchen, 1953; present paper |
| <i>Hippoglossoides elassodon</i> | Epizootic | Flathead Sole | Washington | Wellings, <i>et al.</i> , 1963, 1964, 1965; Chuinard, <i>et al.</i> , 1964 |
| <i>Glyptocephalus zachirus</i> | 3 | Rex Sole | Washington | Chuinard, <i>et al.</i> , 1964; Wellings, <i>et al.</i> , 1965 |
| <i>Microstomus pacificus</i> | Epizootic | Dover Sole | California | Young, 1964 |

part of the growth are present (Pl. figs. 12-14). These elements are PAS-negative.

The hyperplastic epithelium is supported by a stroma of collagenous fibers and vascular elements in which melanin-bearing cells are frequently present (Pl. figs. 7-9). Mucous cells, when present, are usually arranged at the periphery of the growth, or sometimes clustered just below the surface (Pl. figs. 10 & 11). Sloughing and necrosis of the epithelium is evident in some parts of the tumor. The characteristic elements of the tumors are "swollen" epithelial cells, measuring from 15 to 20 microns or about 2 to 3 times the size of the normal cell. These cells have weakly-staining nuclei and vacuolated cytoplasm containing osmiophilic or basophilic granular or filamentous inclusions (Pl. figs. 12-14).

The corium associated with the growths in the sand sole is usually only slightly thickened or edematous (Pl. figs. 7 & 8). Extreme changes, when present, are apparently related to encysted worm parasites (Pl. figs. 15 & 16). The reactions are manifested principally by inflammatory responses and by the development of an extensive, proliferative, angiomatous-like tissue (Pl. figs. 17-19) that extends into the supporting stroma of the hyperplastic epithelium and into the deeper tissues through the intermus-

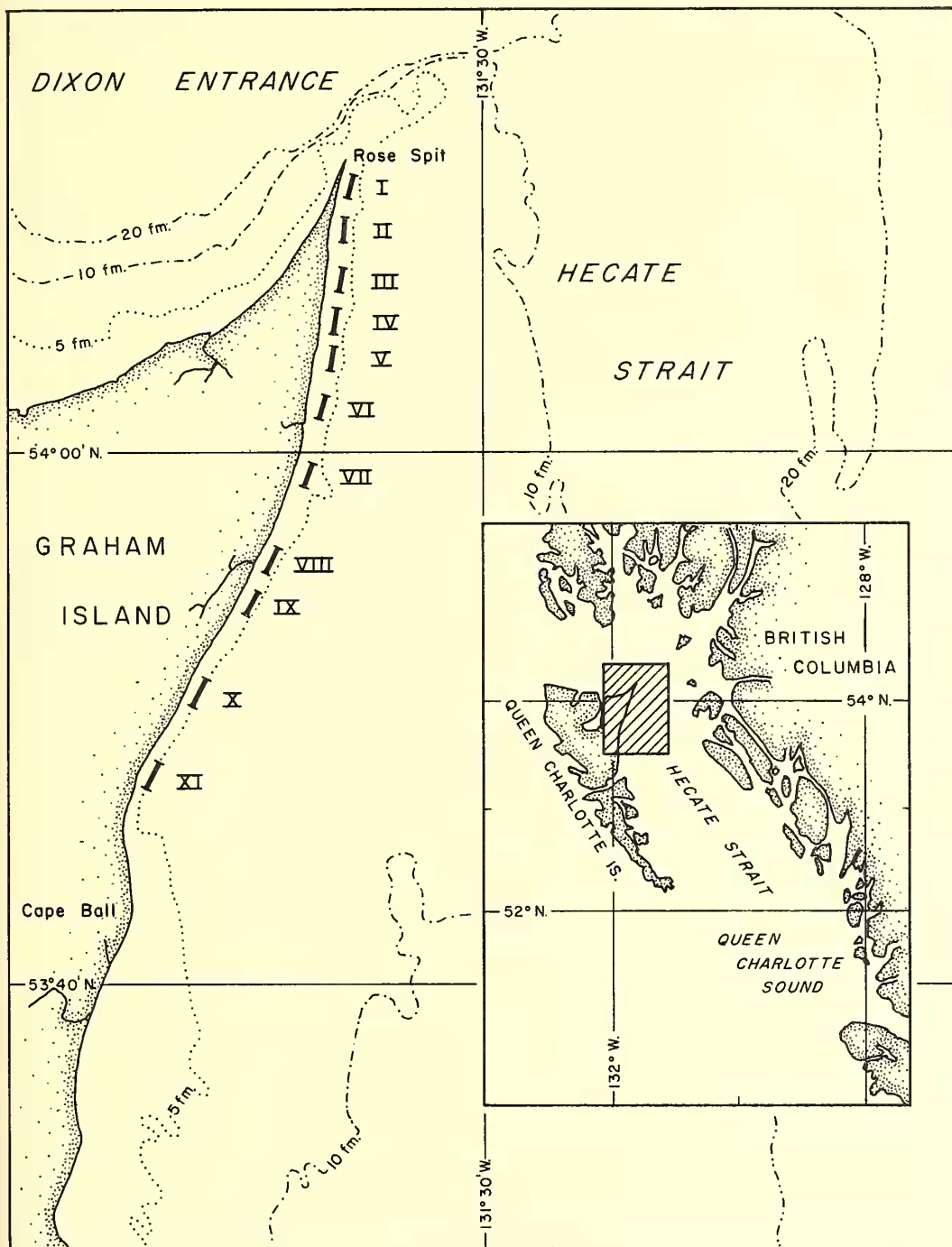
cular pathways, causing destruction of the surrounding tissues (Pl. fig. 20). In addition, periarteritis, angitis and other sclerosing changes of blood vessels, as well as an extensive and striking lymphangiectasis of the cutaneous and sub-muscular lymph vessels, are frequently associated with the lesions (Pl. figs. 21-23).

DISTRIBUTION AND INCIDENCE IN SAND SOLES

The high incidence of epithelial tumors in juvenile sand soles were found in certain stations along the east coast of Graham Island, British Columbia (Text-fig. 1).

Most of the fish affected were 1 to 3 completed years in age. The group measuring from 5 to 17 cm. (mode 10 cm.) consisted of age 1 and 2 fish and the second group from 18 to 26 cm. (mode 22 cm.) consisted mainly of age 3 fish. (Table 3). No individuals of the 0-age group were present in our samples, suggesting, in contrast to other flatfishes, that the growth of the sand sole in the juvenile stages is rather slow.

As noted in Table 2, the incidence of the lesions increased steadily from north to south, being less than 10% in fish caught adjacent to Rose Spit and over 40% in those caught near Cape Ball (Stations X and XI, Text-fig. 1). All these stations were inside the 5-fathom con-



TEXT-FIG. 1. Trawling stations along the eastern shore of Graham Island in Hecate Strait, British Columbia.

tour. In 12 drags made in a section across the deeper part of the bank between the depths of 13 and 18 fathoms, 153 sand soles were collected but none showed signs of the tumor. Text-fig. 2 is a composite graph of all fish taken

along the east coast of the island and clearly shows the relationships between incidence and size of fish. The absence of lesions in fish taken in the drags across the bank in deeper water may be explained by the fact that these fish were

TABLE 2. RECORD OF SAND SOLE CATCHES FROM SHALLOW-WATER SAMPLING STATIONS ALONG THE EAST COAST OF GRAHAM ISLAND, WITH INFORMATION ON TUMOR INCIDENCE

| Station | No. of Drags ¹ | Numbers of fish | | | Tumorous | Percent. Diseased |
|---------|---------------------------|-----------------|----------|---------------------|----------|-------------------|
| | | Caught | Measured | Examined for Tumors | | |
| I | 2 | 26 | 26 | 24 | 2 | 8.3 |
| II | 2 | 72 | 72 | 26 | 2 | 7.7 |
| III | 6 | 337 | 321 | 44 | 7 | 15.9 |
| IV | 2 | 118 | 118 | 45 | 7 | 15.5 |
| V | 5 | 117 | 117 | 36 | 5 | 13.9 |
| VI | 2 | 106 | 106 | 26 | 5 | 19.2 |
| VII | 1 | 45 | 45 | .. | .. | ... |
| VIII | 2 | 307 | 223 | 115 | 28 | 24.4 |
| IX | 2 | 107 | 107 | 64 | 27 | 42.2 |
| X | 3 | 183 | 183 | 143 | 62 | 43.4 |
| XI | 3 | 208 | 208 | 206 | 86 | 41.7 |
| Total | 30 | 1626 | 1526 | 729 | 231 | 31.7% |

¹All drags were of 20 minutes duration, using a small-meshed "shrimp-trawl." Foot rope length: 36 ft.; cod-end mesh size: 1 in. between diagonal knots.

on the average several centimeters larger than those taken along the east coast of Graham Island. The highest incidence was found in the vicinity of Cape Ball where the fish were smaller than those sampled in other areas.

Epithelial tumors were also found occasionally in juvenile rock soles (*Lepidopsetta bilineata*) but none were seen in young butter (*Isopsetta isolepis*) or in lemon (*Parophrys vetulus*) soles of the same area.

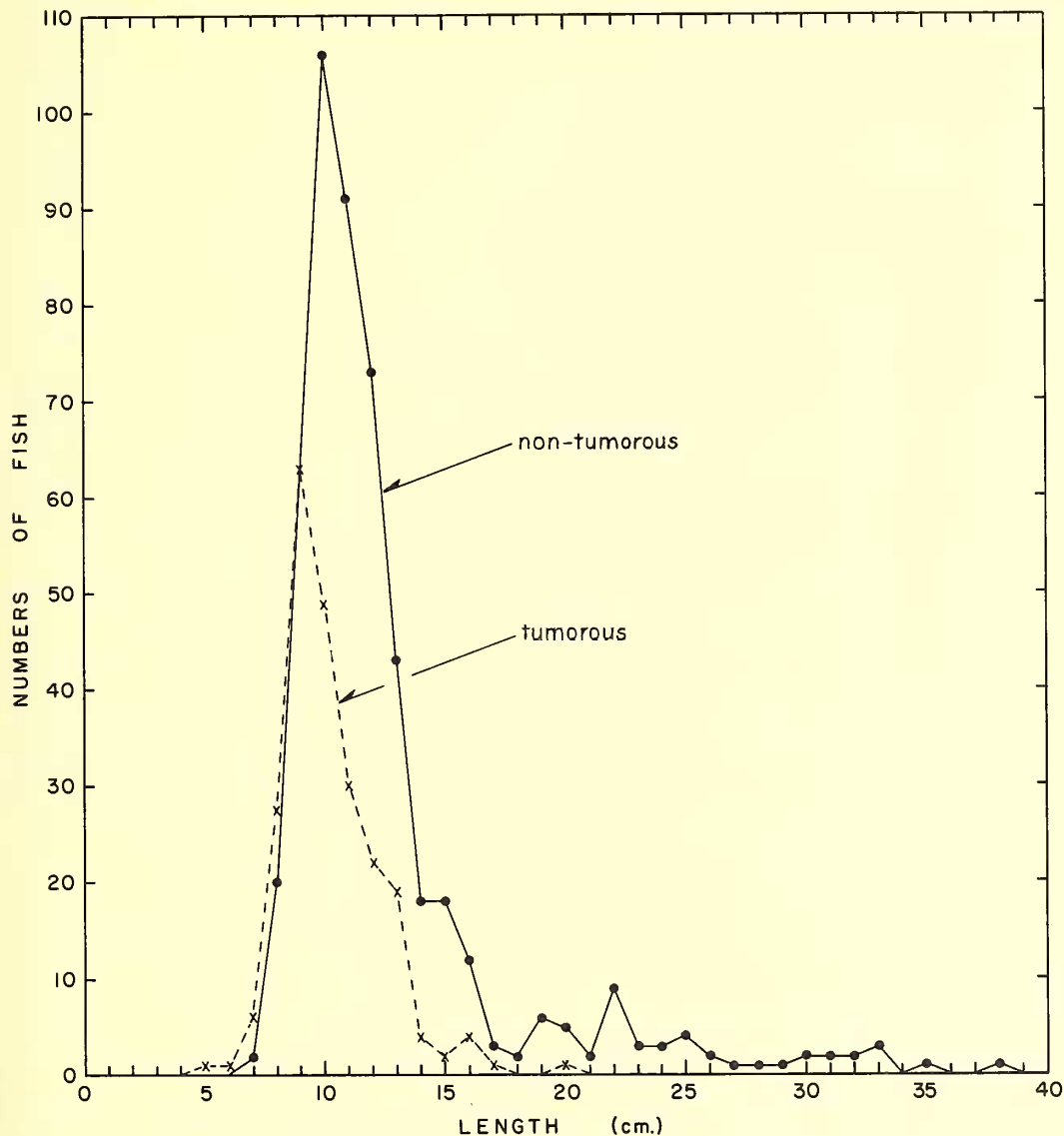
DISCUSSION

Although papillomas have been reported in flatfishes (Johnstone, 1912, 1925; Thomas, 1930; Wellings, *et al*, 1963; Chuinard, *et al*, 1964), the growths in the sand and rock soles from British Columbia in our collection are interpreted as a hyperplastic epidermal disease in which the development of the epithelium may become extensive, sometimes bordering on a papillomatous structure. This suggests that the hyperplasia may represent a pre-neoplastic state. A striking feature in some of the tumors of the sand and rock soles is the development of lymphangiectasis and a proliferative angiomatous-like growth. The inter-relationships of these with each other, with the presence of encysted worm parasites to which these fish are highly susceptible and with the development of the epithelial hyperplasia, are not evident at present. The need for further investigation is indicated by the fact that in mammals angiomatosis and lymphangiectasis are often inter-related, congenital or hereditary in origin, and usually occur in young animals or persons.

The epizootic nature of the epithelial tumors in several species of flatfishes from the Pacific coast, the presence of osmiophilic and basophilic

inclusions reported here in the "swollen" epithelial cells in the sand and rock soles and in the California English sole by Dr. Richard Skahen (Herald & Innes, *pers. comm.*), and the virus-like bodies demonstrated in the flathead soles by electron-microscopy (Wellings & Chuinard, 1964; Wellings *et al*, 1965), strongly indicate an infectious process of viral etiology. The inclusions noted in the present studies are suggestive of the cytomegaloviruses, and reminiscent of the cytoplasmic inclusions seen in the cellular hypertrophy disease (lymphocystis tumors) in European flounders (Weissenberg, 1960), other fishes (Nigrelli & Ruggieri, 1965), and also of those seen in several of the pox and other viral diseases of higher vertebrates (Love, 1959).

The widespread distribution and high incidence of epithelial tumors in flatfishes of the Pacific coastal areas is of considerable economic and biological importance. Apparently, the tumors in fishes of this area were first noted in 1922 by Dr. Carl Hubbs (Scripps Oceanographic Institution, La Jolla) who wrote that he was concerned with the "wart-like dermal swellings" in *Parophrys* in San Francisco Bay and with the possibility that the disease may be correlated with pollution (field notes quoted by Herald & Innes, *pers. comm.*). A survey made in 1951-1953 under the direction of Dr. Herald showed that the disease still existed in San Francisco Bay with an incidence ranging from 16% to 32% in English soles (*Parophrys vetulus*) caught in north bay trawls; a single sample from a south bay "Chinese" shrimp-net catch showed a 6% incidence. No fish less than 50 mm. in length appeared to be affected.



TEXT-FIG. 2. Length-frequency distributions of infected and non-infected sand soles taken at the stations along the east coast of Graham Island.

The epithelial growth in *Parophrys vetulus*, commonly called the English sole in California and Washington and the lemon sole in British Columbia, was studied earlier by Pacis (1932) and by Good (1940). Both of these workers reported lower incidence than in California in fish taken from several beaches at low tide in and around Seattle. Pacis (1932) showed an average incidence of 4.8%, with the tumors occurring most frequently in soles measuring between 9 cm. and 20 cm.; most (73.6%) were in their second year of growth. Good (1940) repeated the studies on English soles from other beaches of Seattle and obtained slightly higher

values (4.7% to 10%), and also showed an apparent seasonal distribution, with the highest incidence occurring in November and December. No diseased fish were found in April and May. Further, he also found that soles under 50 mm. in length (1st year of life) were free of the tumors. Starry flounders (*Platichthys stellatus*) and rock soles (*Lepidopsetta bilineata*) collected at the same time were free of the disease.

Tumors, described as epidermal papillomas, were reported recently in flathead soles, *Hippoglossoides elassodon*, from San Juan Islands and Orcas Island, near Friday Harbor, Washington

TABLE 3. INCIDENCE OF TUMORS IN SAND SOLE, BY SIZE

| Size Group cm. | Numbers of Fish | | |
|-------------------|-----------------|--------------|-------|
| | Tumorous | Non-tumorous | Total |
| 5 | 1 | .. | 1 |
| 6 | 1 | .. | 1 |
| 7 | 6 | 2 | 8 |
| 8 | 28 | 20 | 48 |
| 9 | 63 | 59 | 122 |
| 10 | 49 | 106 | 155 |
| 11 | 30 | 91 | 121 |
| 12 | 22 | 73 | 95 |
| 13 | 19 | 43 | 62 |
| 14 | 4 | 18 | 22 |
| 15 | 2 | 18 | 20 |
| 16 | 4 | 12 | 16 |
| 17 | 1 | 3 | 4 |
| 18 | .. | 2 | 2 |
| 19 | .. | 6 | 6 |
| 20 | 1 | 5 | 6 |
| 21 | .. | 2 | 2 |
| 22 | .. | 9 | 9 |
| 23 | .. | 3 | 3 |
| 24 | .. | 3 | 3 |
| 25 | .. | 4 | 4 |
| 26 | .. | 2 | 2 |
| 27 | .. | 1 | 1 |
| 28 | .. | 1 | 1 |
| 29 | .. | 1 | 1 |
| 30 | .. | 2 | 2 |
| 31 | .. | 2 | 2 |
| 32 | .. | 2 | 2 |
| 33 | .. | 3 | 3 |
| 34 | .. | .. | .. |
| 35 | .. | 1 | 1 |
| 36 | .. | .. | .. |
| 37 | .. | .. | .. |
| 38 | .. | 1 | 1 |
| Total | 231 | 495 | 726 |

(Wellings, *et al*, 1963; Chuinard, *et al*, 1964). The disease was also found occasionally in rock soles and rex soles (*Glyptocephalus zachirus*) from the same area. The incidence in the flathead sole was 5.1% and tumors were found most frequently in the 0-age group.

In northern British Columbia, the tumors are apparently absent from both juvenile and adult lemon (English) soles and butter soles (*Isopsetta isolepis*), but they occur occasionally in the rock sole. In Hecate Strait, for the past 5 years, the average annual Canadian landings of each species of sole have been as follows: 1.6 million lbs. (rock sole); 1.2 million lbs. (lemon sole); 95,000 lbs. (butter sole); 2,000 lbs. (sand sole). The lemon sole is the preferred species. In earlier years catches of lemon sole and rock

sole occasionally have exceeded 5 million lbs. and catches of butter sole have reached about 4 million lbs. (see also, Ketchen, 1956). The sand sole rarely appears in landings in "pure culture." It is so uncommon, at least in Hecate Strait, that by the time it reaches commercial size it is only incidental in landings of other species. It grows to a maximum size of 63 cm., but average size in landings is not much more than 40 cm.

This suggests that the young sand sole is subject to a high natural mortality as a result of the disease³. However, since the pathological evidence indicates that it is a regressive disease, other possible ecological factors may be responsible for what appears to be a reduction of the adult population of sand soles. The diseased fish may be at a disadvantage in competition for food or they may be more susceptible to predation and to abnormal environmental factors. It is quite evident that brood stocks of sand soles are present at all times to sustain a relatively large annual population of at least young fish. Whether or not the susceptible species (lemon or English, flathead, rock, rex, sand and Dover soles) from other areas of the Pacific coast of North America are also subject to an apparent high natural mortality, directly or indirectly related to the epizootics, has not been determined.

SUMMARY

Epizootics in young (1-2 year old) sand soles, *Psettichthys melanosticus*, in Hecate Strait, British Columbia, are characterized by the development of a hyperplastic epidermal disease of the skin, which in some instances is associated with lymphangiectasis and an extensive angiomatous-like proliferative lesion. The epizootiological picture suggests an infectious process, and the cytological evidence indicates that a cytomegalovirus may be the cause.

In British Columbia, the lesions are also found occasionally in the rock sole (*Lepidopsetta bilineata*) but not in the lemon sole (*Parophrys vetulus*) or the butter sole (*Isopsetta isolepis*). All occur in the same general area as the diseased sand soles.

Similar epizootics have been reported in the lemon sole and flathead sole (*Hippoglossoides elassodon*) from Washington, lemon sole from San Francisco Bay and in the Dover sole (*Microstomus pacificus*) from Santa Monica Bay, Cali-

³To some extent the low incidence of sand sole in commercial landings may be attributed to the limited amount of fishing in depths less than 18 fathoms. However, research vessel exploration of these depths have failed to reveal more than scattered occurrence.

fornia. Epithelial tumors have also been reported occasionally in the rex sole (*Glyptocephalus zachirus*) and rock sole from Washington.

It is suggested that the epizootics may be a contributing cause of the high natural mortality in the sand sole in Hecate Strait, British Columbia.

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EXPLANATION OF THE PLATES

PLATE I

- FIG. 1. Epithelial tumors on pigmented side of the sand sole, *Psettichthys melanosticus*, from Hecate Strait, British Columbia. Fish measured 8.7 cm. About 2 ×.
- FIG. 2. Flat, non-pigmented lesion on the eyeless side of another sole measuring 11.9 cm. 1.5 ×.
- FIG. 3. Slightly raised tumor mass on rock sole, *Lepidopsetta bilineata*, from Hecate Strait. About 3.5 ×.

PLATE II

- FIG. 4. Cauliflower-like tumor mass on the pigmented side of English sole (*Parophrys vetulus*) from San Francisco Bay, California. Courtesy Dr. Earl Herald & Department of Ichthyology, California Academy of Sciences. Slightly larger than 2 ×.
- FIG. 5. Section of normal skin of a sand sole, showing the arrangement of the mucous cells. PAS. 300 ×.

PLATE III

- FIG. 6. Section through a transitional area of the skin of the sand sole, showing normal epithelium and enlarged "swollen" epithelial cells characteristic of the hyperplastic area. Hematoxylin-eosin. 1350 ×.
- FIG. 7. Section through fin area of the tumor growth in the sand sole, showing normal and hyperplastic regions of the epidermis. Hematoxylin-eosin. 150 ×.

PLATE IV

- FIG. 8. Area of the tumor of the sand sole, showing an excessive development of the epithelium but paucity of corial tissue. Note papillary arrangement, suggesting a papillomatous-like structure. Azan. 52 ×.
- FIG. 9. Hyperplastic epithelium in sand sole, showing melanin-bearing cells in supporting stroma. Giemsa. 150 ×.

PLATE V

- FIG. 10. Hyperplastic epithelium in sand sole, showing distribution of mucous elements in the peripheral zone. PAS. 150 ×.
- FIG. 11. Clusters of mucous cells in the deeper layers of the hyperplastic epithelium in rock sole. PAS. 300 ×.

PLATE VI

- FIG. 12. Details of "swollen" epithelial cells characteristic of the tumors in sand sole. No

mitotic figures were seen; the nucleus is weakly staining, the nucleolus slightly swollen and cytoplasm vacuolated. Note granular inclusions. Heidenhain's hematoxylin. 1350 ×.

- FIG. 13. Higher magnification of another area, demonstrating the cytoplasmic inclusions shown in Fig. 12. Heidenhain's hematoxylin. 2500 ×.

PLATE VII

- FIG. 14. Similar cells in rock sole. The central cell shows filamentous inclusions. Heidenhain's hematoxylin. 1350 ×.
- FIG. 15. Corial region of the tumor, showing encysted worm parasites in sand sole. Azan. 150 ×.

PLATE VIII

- FIG. 16. Another area of the same fish with encysted metacercaria (Echinostome). Note development of connective tissue. Hematoxylin-eosin. 600 ×.
- FIG. 17. Angiomatous-like reaction in sand sole infected with the parasites. Hematoxylin-eosin. 600 ×.

PLATE IX

- FIG. 18. Similar reaction associated with the epithelial hyperplasia in the rock sole. Hematoxylin-eosin. 300 ×.
- FIG. 19. Section of tumor in sand sole, showing the relation of the angiomatous-like tissue to the supporting stroma of the epithelial hyperplasia. Hematoxylin-eosin. 300 ×.

PLATE X

- FIG. 20. Proliferation of the angiomatous-like tissue shown in Fig. 17 into intermuscular pathways. Hematoxylin-eosin. 150 ×.
- FIG. 21. Periarteritis in another area of the same section shown in Fig. 20. Hematoxylin-eosin. 600 ×.

PLATE XI

- FIG. 22. Lymphangiectasis in corial region associated with the epithelial hyperplasia in the rock sole. Hematoxylin-eosin. 150 ×.
- FIG. 23. Lymphangiectasis in submuscular area of sand sole with hyperplastic epithelium and angiomatous-like proliferative tissue shown in Fig. 17. Hematoxylin-eosin. 600 ×.